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Effect of Latrunculin-B on Outflow Facility in Monkeys

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Latrunculin-B (LAT-B), a macrolide derived from the marine sponge *Latrunculia magnifica*, sequesters monomeric G-actin, leading to the disassembly of actin filaments in cultured cells. In this study, we determined the effect of LAT-B on outflow facility in living monkeys. Total outflow facility was measured by 2-level constant pressure perfusion of the anterior chamber (AC) before and immediately after AC exchange infusion or 2 hr after topical application of LAT-B or vehicle. Both AC exchange infusion and topical application of LAT-B dose- and time-dependently increased outflow facility by two- to four-fold. Those findings suggest that pharmacological disorganization of the actin cytoskeleton in the trabecular meshwork by specific actin inhibitors such as LAT-B may be a useful anti-glaucoma strategy.
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Key words: aqueous humor outflow facility; latrunculin B; monkey eye; trabecular meshwork.

1. Introduction

Latrunculins, macrolides isolated from the marine sponge *Latrunculia magnifica*, sequester monomeric G-actin, leading to the disassembly of actin filaments (Coué et al., 1987; Lyubimova, Bershadsky and Ben-Ze'ev, 1997; Spector et al., 1983). Latrunculin A (LAT-A) causes a reversible dose- and incubation time-dependent destruction of actin bundles in several types of cultured cells (Coué et al., 1987; Lyubimova, Bershadsky and Ben-Ze'ev, 1997; Spector et al., 1983). LAT-A significantly increases outflow facility in living monkeys, perhaps by disrupting the actin cytoskeleton in trabecular meshwork (TM) cells, in turn relaxing the meshwork and/or separating cell–cell and cell–extracellular matrix adherens junctions within the meshwork (Peterson et al., 1999). This suggests that as actin-disrupting agents latrunculins may have potential as anti-glaucoma medication. However, LAT-A also disturbs anterior segment barrier functions, increasing corneal endothelial permeability and anterior chamber (AC) protein concentration, and probably increasing aqueous humor flow (AHF), ciliary epithelial and iridovascular endothelial permeability as well, early after topical drug administration (Peterson et al., 1998). Latrunculin B (LAT-B) has similar structure and actin-disrupting activity in cultured cells to LAT-A (Coué et al., 1987; Lyubimova, Bershadsky and Ben-Ze'ev, 1997; Spector et al., 1983), but LAT-B's effect on the morphology and actin organization in hamster fibroblasts requires higher concentrations than LAT-A (Spector et al., 1989). Moreover, LAT-B, but not LAT-A, is slowly inactivated by an as yet unknown serum component so that after 48 hr of exposure to a maximal LAT-B dose, cells completely recover, exhibiting a well-developed system of stress fibers (Spector et al., 1989). In living monkeys, LAT-B has a milder effect on corneal endothelial permeability than LAT-A, and has essentially no effect on AHF (Peterson et al., 1998). Thus LAT-B may be 'gentler' to the ocular tissues. Although LAT-B has recently been shown to increase outflow facility in enucleated porcine eyes (Epstein, Rowlette and Roberts, 1999), it was not known if it would have the same effect in the live monkey eye. Therefore, it seemed worthwhile to study LAT-B's effects on outflow facility in living monkeys.

2. Materials and Methods

Live Monkeys

Normal adult cynomolgus (*Macaca fascicularis*) monkeys were anesthetized with intramuscular (i.m.) ketamine (10 mg kg^{-1}) followed by i.m. (35 mg kg^{-1}) or intravenous (i.v.; 15 mg kg^{-1}) pentobarbital sodium, supplemented with 10 mg kg^{-1} i.v. injections as needed. All experiments were conducted in accordance with UW and NIH guidelines, and with the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research.

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The University of Wisconsin and the Weizmann Institute of Science hold a patent related to this manuscript, accordingly, Drs Kaufman (UW) and Geiger (WIS) have a proprietary interest. Alcon Laboratories, Inc., Fort Worth, TX, U.S.A. has paid a fee to UW and WIS for an option to license this technology, and has provided some financial support in this general area. Dr Kaufman has served as a paid consultant on unrelated issues for Alcon and several other pharmaceutical corporations.

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Chemicals and Drug Preparation

LAT-B was obtained from Calbiochem-Novabiochem Int., Inc. (La Jolla, CA, U.S.A.) and stored as a 2 mM stock solution in DMSO (dimethyl sulfoxide; Sigma Chemical Co., St. Louis, MO, U.S.A.) at −20°C. LAT-B solutions for the 0·02, 0·06, 0·2 and 2 μM exchange perfusions were formulated as 0·2, 0·6, 2 or 20 μl of 2 mM LAT-B stock solution, 49·8, 49·4, 48 or 30 μl of DMSO and 19·95 ml of Bárány's mock aqueous humor solution (Bárány, 1964). The vehicle for all exchange perfusions was formulated as 50 μl DMSO and 19·95 ml Bárány's solution (0·25% DMSO). The LAT-B and vehicle solutions for topical application (200 μM and 500 μM LAT-B, 10% and 25% DMSO) were formulated, respectively, as 3 μl of 2 mM LAT-B stock solution or DMSO, +27 μl Bárány's solution; or 11·25 μl 2 mM LAT-B stock solution or DMSO, +33·75 μl Bárány's solution.

Outflow Facility

Total outflow facility was determined by 2-level constant pressure perfusion of the anterior chamber (AC) with Bárány's mock aqueous humor (Bárány, 1964), correcting for the internal resistance of the perfusion apparatus as appropriate. Most monkeys had undergone prior perfusions but not within the preceding 5–6 weeks, all were free of AC cells and flare by slit-lamp biomicroscopy.

Exchange Infusion The AC of both eyes was cannulated with a branched needle connected to a reservoir and pressure transducer and an unbranched needle with tubing clamped off. Baseline facility measurements were taken for 35–45 min. The clamped tubing from the unbranched needle was then connected to syringes containing drug (0·02, 0·06, 0·2 or 2 μM LAT-B) or vehicle (0·25% DMSO). The syringes were placed in a variable speed infusion pump and the tubing previously leading to the reservoir was disconnected from the reservoir and opened to air as a temporary outflow line, allowing infusion of 2 ml of solution through the AC over 10–15 min, while maintaining IOP at ∼15 mmHg by adjusting the height (e.g. 15–16 cm higher than the eye) of the end of the outflow tubing. The reservoirs were emptied and re-filled with the same solution being perfused through the eye. The 'temporary outflow' tubing was reconnected to the reservoir and the syringe tubing was clamped again, allowing infusion from the reservoir into the eye. Outflow facility measurements were immediately taken for 90 min.

Topical Eyedrops The AC of both eyes was cannulated with a branched needle as above, after which baseline facility measurements were taken for 35–45 min. With reservoirs closed, 2 × 5 μl drops of 200 μM (∼0·8 μg) or 4 × 5 μl drops of 500 μM (∼4·0 μg) LAT-B were given to one eye of the prone monkey, allowing 60–90 sec between drops. The drops were placed at the superior limbus and allowed to flow down the cornea, after which the lower lid was lifted two or three times. Vehicle (10 or 25% DMSO, respectively) was given simultaneously to the opposite eye in a similar manner. The doses were chosen to give 0·2 μM and 1 μM LAT-B concentrations, respectively, in the ∼100 μl monkey AC (Erickson-Lamy et al., 1984), assuming 1% penetration and no initial drug loss from the AC (Asseff et al., 1973; Harris, 1968; Janes and Stiles, 1959). The perfusion system remained closed for 2 hr, after which facility measurements were taken for 90 min. Biomicroscopy was performed by an ophthalmologist before and 3, 7 and 14 days after drug administration.

Statistical Analysis

Data are presented as means ± S.E.(M.) for *n* eyes or animals. Pre- or post-LAT-B treated vs contralateral control; post-LAT-B or post-vehicle vs ipsilateral baseline; and baseline corrected post-LAT-B treated vs control comparisons were made using the two-tailed paired *t*-test for differences vs 0·0 or ratios vs 1·0.

3. Results

Outflow Facility

Exchange infusion of 0·02–2·0 μM LAT-B produced a dose- and time-dependent facility increase, with 2 μM LAT-B infusion increasing facility by an average of 456 ± 128% ($n = 5$, $P < 0{\cdot}025$) over a 90 min period, correcting for baseline differences and control eye washout (Fig. 1 and Table I).

Topical application of LAT-B produced a dose- and time-dependent facility increase of 123 ± 67% ($n = 5$, P = NS) and 272 ± 45% ($n = 5$, $P < 0{\cdot}005$) for the 0·8 and 4·0 μg doses respectively, averaged over a 90 min period (Fig. 2 and Table II). Biomicroscopy 3 days after topical drug administration and AC perfusion showed no adverse effects at the 0·8 μg dose. At the 4·0 μg dose, two drug-treated eyes exhibited a small clot of blood in the AC and two drug-treated eyes and one vehicle-treated eye exhibited a few keratic precipitates on day 3 after perfusion. By day 7 all eyes appeared biomicroscopically normal.

To determine the initial LAT-B effect, we compared the first facility value upon restarting the perfusion after drug administration to the final baseline value obtained just prior to drug administration (PRx_i/BL_f in Table III). Both exchange infusion and topical eyedrop administration caused a dose-dependent initial facility increase, with the initial post-drug value increasing over the final baseline value by 379 ± 125% [$n = 5$, $P < 0{\cdot}05$; Table III(D)] following 2 μM LAT-B exchange infusion and by 173 ± 73% [$n = 5$, $P < 0{\cdot}1$; Table III(F)] 2 hr after topical 4·0 μg LAT-B. Baseline facility 5–13 weeks after intra-

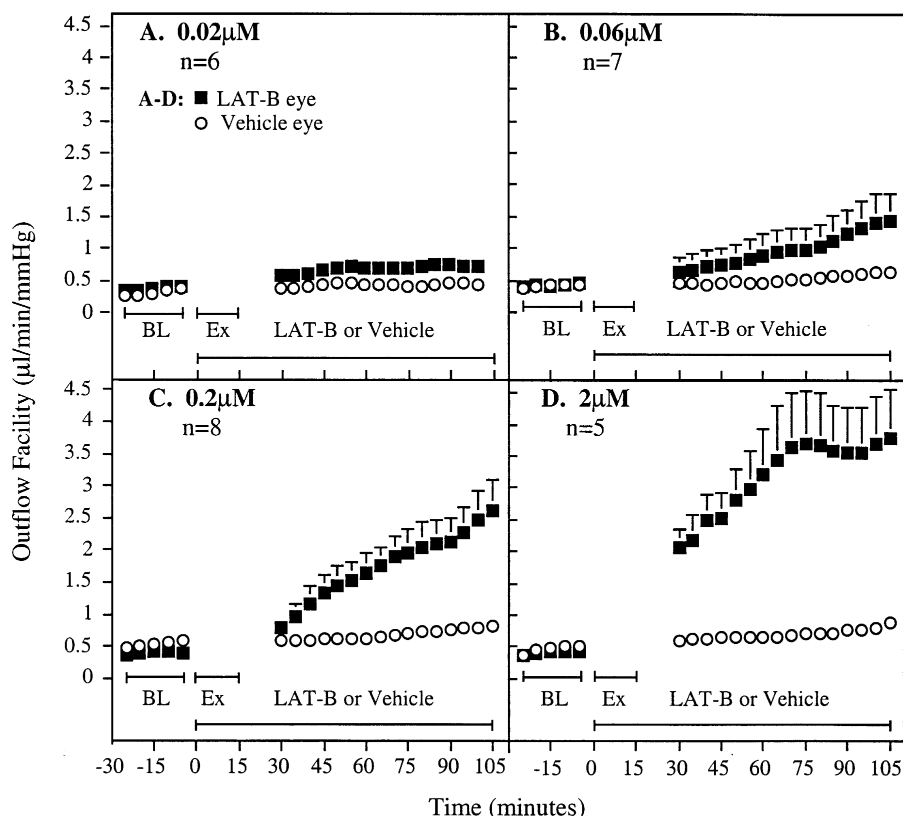

FIG. 1. Outflow facilities vs time for LAT-B exchange protocols. Each data point is the mean ± S.E.(M.) of the facility readings at that time (some error bars are smaller than the symbols) for n monkeys, each contributing one LAT-B-treated and one vehicle-treated eye. Anterior chamber (AC) exchange begins at time 0. BL = baseline, Ex = 2 ml exchange of AC with LAT-B or vehicle.

TABLE I

Intracameral LAT-B exchange, facilities by 30 min intervals

LAT-B Dose			Facility (μl min^{-1} mmHg^{-1}) LAT-B	Veh	LAT-B/Veh	(LAT-B/BL)/ (Veh/BL)
(A)	0·02 μM	BL	0·38 ± 0·06	0·31 ± 0·05	1·30 ± 0·18	
	(n = 6)	0–30 min	0·64 ± 0·11	0·44 ± 0·06	1·61 ± 0·32	1·19 ± 0·14
		30–60 min	0·70 ± 0·14	0·42 ± 0·06	1·84 ± 0·40*	1·34 ± 0·19
		60–90 min	0·73 ± 0·14	0·45 ± 0·06	1·81 ± 0·37*	1·33 ± 0·14*
(B)	0·06 μM	BL	0·43 ± 0·13	0·41 ± 0·10	1·10 ± 0·25	
	(n = 7)	0–30 min	0·77 ± 0·27	0·46 ± 0·08	1·59 ± 0·24*	1·51 ± 0·18†
		30–60 min	0·98 ± 0·34	0·50 ± 0·10	1·79 ± 0·25‡	1·81 ± 0·20§
		60–90 min	1·30 ± 0·43	0·61 ± 0·11	1·97 ± 0·28‡	2·05 ± 0·28§
(C)	0·2 μM	BL	0·39 ± 0·04	0·54 ± 0·12	0·83 ± 0·10	
	(n = 8)	0–30 min	1·29 ± 0·26	0·61 ± 0·11	2·48 ± 0·62†	2·99 ± 0·60‡
		30–60 min	1·86 ± 0·32	0·68 ± 0·10	2·98 ± 0·60‡	3·73 ± 0·63‖
		60–90 min	2·31 ± 0·42	0·78 ± 0·09	2·95 ± 0·40‖	3·88 ± 0·64‖
(D)	2 μM	BL	0·41 ± 0·08	0·46 ± 0·09	0·90 ± 0·07	
	(n = 5)	0–30 min	2·60 ± 0·45	0·64 ± 0·08	4·34 ± 1·01†	4·79 ± 0·97‡
		30–60 min	3·54 ± 0·81	0·69 ± 0·10	5·64 ± 1·72*	6·16 ± 1·64†
		60–90 min	3·64 ± 0·73	0·79 ± 0·11	5·00 ± 1·37†	5·47 ± 1·29†

BL = Baseline, Veh = Vehicle-treated eye, LAT-B = LAT-B-treated eye. Data are mean ± S.E.(M.) for n animals, each contributing one LAT-B-treated and one vehicle-treated eye. * $P < 0·1$, † $P < 0·05$, ‡ $P < 0·02$, § $P < 0·01$, ‖ $P < 0·005$ for ratios different from 1·0 by the two-tailed paired t-test.

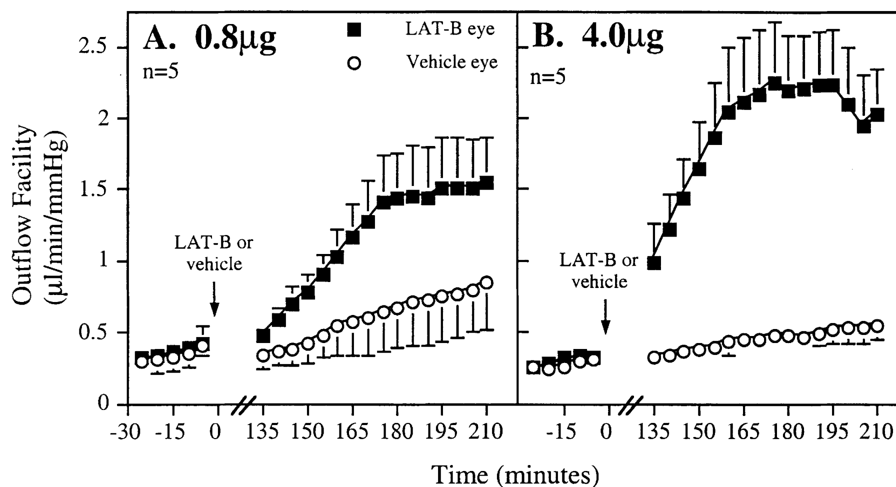

FIG. 2. Outflow facilities vs time for LAT-B topical protocols. Each data point is the mean ± S.E.(M.) of the facility readings at that time (some error bars are smaller than the symbols) for *n* monkeys, each contributing one LAT-B-treated and one vehicle-treated eye. Drug administration begins at time 0.

TABLE II

Topical LAT-B, facilities by 30 min intervals

LAT-B Dose			Facility (μl min^{-1} mmHg^{-1}) LAT-B	Veh	LAT-B/Veh	(LAT-B/BL)/(Veh/BL)
(A)	0·8 μg	BL	0·37 ± 0·07	0·34 ± 0·10	1·18 ± 0·11	
	2 hr wait	0–30 min	0·80 ± 0·13	0·44 ± 0·14	2·30 ± 0·65	1·84 ± 0·33
	(n = 5)	30–60 min	1·35 ± 0·30	0·64 ± 0·27	3·37 ± 1·40	2·59 ± 0·81
		60–90 min	1·50 ± 0·34	0·77 ± 0·31	3·14 ± 1·47*	2·37 ± 0·87
(B)	4·0 μg	BL	0·31 ± 0·03	0·27 ± 0·04	1·18 ± 0·16	
	2 hr wait	0–30 min	1·64 ± 0·34	0·38 ± 0·07	4·49 ± 1·12†	3·68 ± 0·47‖
	(n = 5)	30–60 min	2·19 ± 0·42	0·46 ± 0·07	4·74 ± 0·86‡	4·03 ± 0·46‖
		60–90 min	2·11 ± 0·36	0·52 ± 0·10	4·22 ± 0·67§	3·68 ± 0·52§

BL = Baseline, Veh = Vehicle-treated eye, LAT-B = Lat-B-treated eye. Data are mean ± S.E.(M.) for n animals, each contributing one LAT-B-treated and one vehicle-treated eye. *$P < 0·1$, †$P < 0·05$, ‡$P < 0·02$, §$P < 0·01$, ‖$P < 0·005$ for ratios different from 1·0 by the two-tailed paired t-test.

cameral or topical LAT-B was not significantly different from the baseline immediately prior to receiving LAT-B (Fig. 3 and Table IV).

4. Discussion

Similar to LAT-A (Peterson et al., 1999), LAT-B disrupts the actin cytoskeleton in cultured cells (Coué et al., 1987; Lyubimova, Bershadsky and Ben-Ze'ev, 1997; Spector et al., 1983) and increases outflow facility in living monkeys, further supporting a relationship between alteration of the actin cytoskeleton in TM cells and decreased flow resistance in the conventional drainage pathway. Since the 0·2 μM intracameral dose and the 4·0 μg topical dose of LAT-B produce similar facility elevations to the 2 μM intracameral dose and the 42 μg topical dose of LAT-A (Peterson et al., 1999), LAT-B appears to be at least ten-fold more potent than LAT-A in the live monkey TM. Additionally, the initial facility value upon restarting the perfusion immediately after AC exchange or 2 hr after topical application of maximal doses of LAT-B is substantially elevated when compared to the final baseline measurement (Figs 1, 2 and Table III). In contrast, even maximally effective doses of LAT-A produce less initial facility increase under the same experimental conditions, only upon continued perfusion is there such a significant decrease in flow resistance (Peterson et al., 1999). Topical LAT-A and B significantly reduce IOP in normal monkeys, indicating that both drugs reduce resistance in the drainage pathway at normal flow rates (Kaufman et al., 1998). However, the pressure- and flow-dependence of their facility-increasing effect indicates that the higher pressure and flow rate induced by external perfusion, as opposed to lower endogenous AHF,

TABLE III

Intracameral and topical LAT-B, initial facilities

		Time	Facility (μl min^{-1} mmHg^{-1}) or Facility Ratio		
			LAT-B	Veh	LAT-B/Veh
(A)	0·02 μM	BL_f	0·40 ± 0·07	0·37 ± 0·05	1·14 ± 0·17
	Ex	PRx_i	0·57 ± 0·09	0·37 ± 0·05	1·70 ± 0·33*
	(n = 6)	PRx_f	0·72 ± 0·15	0·44 ± 0·07	1·85 ± 0·41*
		PRx_i/BL_f	1·47 ± 0·14‡	1·02 ± 0·08	1·45 ± 0·11‖
		PRx_f/BL_f	1·86 ± 0·30†	1·21 ± 0·07†	1·56 ± 0·23*
		PRx_f/PRx_i	1·26 ± 0·14	1·21 ± 0·09*	1·06 ± 0·11
(B)	0·06 μM	BL_f	0·46 ± 0·14	0·43 ± 0·10	1·15 ± 0·28
	Ex	PRx_i	0·63 ± 0·23	0·48 ± 0·11	1·29 ± 0·26
	(n = 7)	PRx_f	1·43 ± 0·44	0·64 ± 0·12	2·09 ± 0·31§
		PRx_i/BL_f	1·31 ± 0·09§	1·16 ± 0·11	1·16 ± 0·09
		PRx_f/BL_f	3·52 ± 0·72§	1·61 ± 0·10 #	2·16 ± 0·36§
		PRx_f/PRx_i	2·63 ± 0·43‖	1·43 ± 0·11‖	1·83 ± 0·23§
(C)	0·2 μM	BL_f	0·41 ± 0·04	0·59 ± 0·12	0·78 ± 0·10*
	Ex	PRx_i	0·78 ± 0·13	0·58 ± 0·09	1·64 ± 0·46
	(n = 8)	PRx_f	2·60 ± 0·49	0·81 ± 0·09	3·18 ± 0·43¶
		PRx_i/BL_f	2·07 ± 0·44†	1·02 ± 0·06	2·01 ± 0·34‡
		PRx_f/BL_f	6·51 ± 1·16¶	1·55 ± 0·20†	4·36 ± 0·66¶
		PRx_f/PRx_i	3·38 ± 0·45¶	1·49 ± 0·14‖	2·40 ± 0·37‖
(D)	2·0 μM	BL_f	0·43 ± 0·09	0·51 ± 0·09	0·94 ± 0·07
	Ex	PRx_i	2·07 ± 0·28	0·59 ± 0·06	3·63 ± 0·58§
	(n = 5)	PRx_f	3·80 ± 0·75	0·88 ± 0·13	4·69 ± 1·29†
		PRx_i/BL_f	5·70 ± 1·40†	1·22 ± 0·10*	4·79 ± 1·25†
		PRx_f/BL_f	9·76 ± 2·05§	1·77 ± 0·11¶	5·73 ± 1·37†
		PRx_f/PRx_i	1·78 ± 0·14‖	1·47 ± 0·09‖	1·24 ± 0·16
(E)	0·8 μg	BL_f	0·43 ± 0·09	0·41 ± 0·13	1·14 ± 0·08
	Top	PRx_i	0·47 ± 0·08	0·34 ± 0·10	1·65 ± 0·38
	(n = 5)	PRx_f	1·54 ± 0·33	0·84 ± 0·32	2·82 ± 1·22
		PRx_i/BL_f	1·16 ± 0·11	0·87 ± 0·06*	1·36 ± 0·16*
		PRx_f/BL_f	3·78 ± 0·70§	2·00 ± 0·31†	2·12 ± 0·64
		PRx_f/PRx_i	3·41 ± 0·73†	2·33 ± 0·35§	1·50 ± 0·28
(F)	4·0 μg	BL_f	0·32 ± 0·04	0·31 ± 0·05	1·09 ± 0·11
	Top	PRx_i	0·98 ± 0·27	0·27 ± 0·05	3·19 ± 1·16
	(n = 5)	PRx_f	2·03 ± 0·31	0·54 ± 0·10	3·92 ± 0·58‖
		PRx_i/BL_f	3·03 ± 0·94*	1·04 ± 0·10	2·73 ± 0·73*
		PRx_f/BL_f	6·19 ± 0·48 #	1·83 ± 0·31*	3·69 ± 0·55‖
		PRx_f/PRx_i	3·16 ± 1·08	1·77 ± 0·25†	1·80 ± 0·53

BL = Baseline facility; Veh = Vehicle-treated eye; Ex = exchange infusion; Top = topical administration; LAT-B = LAT-B-treated eye; PRx = Facility post-ipsilateral LAT-B and contralateral vehicle administration; $_i$ = first value of measurement period, $_f$ = final value of measurement period. Data are mean ± S.E.(M.) for n animals, each contributing one LAT-B-treated and one vehicle-treated eye. * $P < 0·1$, † $P < 0·05$, ‡ $P < 0·025$, § $P < 0·02$, ‖ $P < 0·01$, ¶ $P < 0·005$, # $P < 0·001$ for ratios ≠ 1·0 by the two-tailed paired t-test.

further destabilizes TM cell junctions and overall TM architecture to reduce flow resistance more substantially. Under this scenario, such actin-disrupting agents may be more effective in glaucoma patients with elevated IOP and a greater trans-trabecular pressure gradient, especially, if they are not receiving secretory suppressants. The current data suggest that the higher doses of LAT-B may render the TM architecture more unstable even at normal flow rates, so that LAT-B might also be more effective than LAT-A in normal-pressure glaucoma or in glaucoma with pressure already reduced by other agents. Moreover, the fact that the baseline facility several weeks after LAT-B is similar to that before the drug administration indicates that LAT-B, similar to LAT-A (Peterson et al., 1999), induces a transient alteration in cytoskeletal organization and cell adhesions rather than irreversible toxicity in the TM cells. However, it is not clear whether LAT-A or LAT-B will produce longer-lasting facility increase.

The results in vivo contrast to the situation in vitro, where lower doses of LAT-A produce the same maximal changes in cell morphology as higher doses of LAT-B (Spector et al., 1989). The only structural difference between LAT-A and LAT-B is in the macrolide (Spector et al., 1989), a large apolar part of the molecule that is probably important for permeation into the cell, LAT-A has a diene moiety vs the monoene moiety of LAT-B (Spector et al., 1986, 1989). Additionally, some factor may be present in the

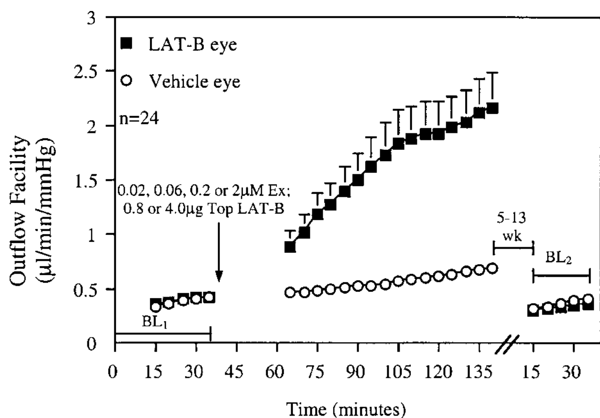

FIG. 3. Baseline (BL) outflow facility, post-drug facility for 90 min after different concentrations of intracameral or topical LAT-B or vehicle, and baseline facility from the following perfusion 5–13 weeks later vs time. Each data point is the mean ± S.E.(M.) facility at that time (some error bars are smaller than the symbols) for *n* monkeys that received only one dose of LAT-B following BL_1 and did not receive any other treatment between BL_1 and BL_2. Each monkey contributed one LAT-B treated and one vehicle-treated eye. BL_1 = baseline before LAT-B or vehicle administration, BL_2 = baseline at the following perfusion, Ex = AC exchange infusion. Top = topical administration.

TABLE IV

Comparison of baseline outflow facilities immediately before and 5–13 weeks after LAT-B

	Facility (μl min^{-1} mmHg^{-1})		
	LAT-B	Veh	LAT-B/Veh
BL_1	0·40 ± 0·04	0·39 ± 0·04	1·12 ± 0·09
BL_2	0·33 ± 0·03	0·36 ± 0·03	1·01 ± 0·08
BL_2/BL_1	0·90 ± 0·05	1·03 ± 0·10	0·96 ± 0·06

LAT-B = LAT-B-treated eye, Veh = Vehicle-treated eye, BL_1 = Baseline immediately before intracameral or topical LAT-B/vehicle administration; BL_2 = Baseline at the following perfusion 5–13 weeks after LAT-B. Data are mean ± S.E.(M.) for 24 animals that received only one dose of different concentrations of intracameral or topical LAT-B following BL_1 and did not receive any other treatment between BL_1 and BL_2. Each monkey contributed one LAT-B-treated and one vehicle-treated eye.

TM cells or the AC of the living monkey that is not present in the in vitro cell types and experimental conditions previously reported, favoring LAT-B's penetration into the meshwork cells. In vitro, an unknown component in serum-containing growth medium slowly inactivates the effect of LAT-B but not LAT-A on the cytoskeleton (Spector et al., 1989). However, the tight-junctioned ciliary epithelium and irido-vascular endothelium largely exclude serum proteins from the aqueous humor (Krupin and Civan, 1996), LAT-B has at most a minimal and transient effect on AC protein concentration (Peterson et al., 1998), and the perfusand used in our experiments is serum-free (Bárány, 1964). Therefore, LAT-B may avoid inactivation in our in vivo experiments. Better cellular penetration and/or reduced inactivation would both favor more rapid attainment of higher concentrations in the TM cells of the living eye. It is also possible that the TM cells may be more sensitive to LAT-B than LAT-A, that the structural changes in the TM following LAT-B may be partially different from those induced by LAT-A, or that some additional non-cytoskeletal action may enhance LAT-B's facility effect. Any or all of these factors, or other pharmacodynamic differences and/or tissue specificities, could contribute to the differential potency in vivo. Whatever the reasons, it is noteworthy from a potential clinical perspective that LAT-B had less effect on AHF and corneal endothelial permeability than LAT-A in the monkey eye (Peterson et al., 1998), despite having an equal or greater effect on facility.

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